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Extraction of antioxidant polyphenolic compounds from peanut skin using water-ethanol at high pressure and temperature conditions

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ABSTRACT

This study evaluates the feasibility of using sub-critical water extraction (SWE) processes to obtain antioxidant phenolic compounds from peanut skins. Theoretical models were tested against experimental data in order to optimize extraction conditions and antioxidant activity of target compounds. The maximum concentration of total phenolics was achieved by using 60.5% ethanol as co-solvent, at 220 °C extraction temperature and 7 g/min solvent flow. Under these extraction conditions, a large number and variety of polyphenols were identified. Phenolic profile was dominated largely by monomeric and condensed flavonoids, particularly procyanidin and proanthocyanidin oligomers. Extracts obtained under those conditions also gave the best radical scavenging capacities, which were higher to those reached by using a synthetic antioxidant (BHT, butylhydroxytoluene). Kinetic studies showed a high extraction rate of polyphenols until the first 30 min of extraction, and it was in parallel with the highest antioxidant activity.

1. Introduction

Peanut is a major source of oil and protein, ranked as the second-most important grain legume cultivated, and the fourth largest edible oilseed crop in the world. It is mostly used in confectionaries, snacks and for edible oil production. In addition to high lipid and protein contents (45–55% and 22–30% of the nut weight, respectively), peanuts contain a vast array of compounds having important biological properties [1–3]. These include primarily crude fiber, tocopherols and other vitamins (mainly niacin, riboflavin and pantothenic acid), sterols, and several phenolic and polyphenolic substances. These latter are found at the highest concentration in the seed coat (the skin or pellicle surrounds the kernel).

The peanut skin is a by-product of peanut blanching operations with the only current market being low value animal feed applications [3]. Several studies suggest a potential to produce nutraceutical ingredients from peanut skin extracts. The main classes of natural phenolics (phenolic acids, flavonoids and stilbenes), including various procyanidins and proanthocyanidins, have been found in peanut skin, and several studies have reported their antioxidant properties. For instance, Yu et al. [4] isolated catechins and procyanidin oligomers from chemically purified peanut skin extracts, and demonstrated higher free radical scavenging capacity than Trolox and Vitamin C. Similarly, Larrauri et al. [5] showed that peanut skin extracts – purified by means of different organic solvents, and composed mainly by phenolic acids, flavonoids, procyanidin and proanthocyanidin dimers – display strong scavenging capacity toward different synthetic free radicals. Oldoni et al. [6] used bioassay-guided fractionation to isolate antioxidant compounds from peanut skin. They identified two monomeric proanthocyanidins as the main bioactive components. Proanthocyanidin A1 showed better antiradical activity than that of BHT, a synthetic antioxidant. Regarding applications in food systems, it has been found that peanut skin extracts can reduce oxidative degradation of sunflower oil Larrauri et al. [5]. Other studies reveal the potential of peanut polyphenolics to enhance the chemical stability and sensory properties, and to extend the shelf-life of processed foods [7,8].

Peanut skin extracts, such as those mentioned previously, have been traditionally obtained by means of conventional solid-liquid extraction

Abbreviations: BHT, butylhydroxytoluene; DB, dry basis; DPS, defatted peanut skin; GAE, gallic acid equivalent; QE, quercetin equivalent; PSE, peanut skin extract; RSC, radical scavenging capacity; SWE, sub-critical water extraction; TFC, total flavonoid content; TPC, total phenol content

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techniques (maceration, Soxhlet) using different organic solvents, such as methanol, ethanol, acetone, diethyl ether, and ethyl acetate, among others. Due to their hydrogen-bonding ability, which is crucial for the extraction of phenolics, those solvents may provide high extraction yields, but poor selectivity; so, further purification steps are often required. In addition, most of the organic solvents are generally prohibited by food regulations throughout the world.

In recent years, sub-critical or superheated water extraction (SWE) (also named as pressurized hot water extraction) has emerged as a promising green solvent extraction method for different kinds of natural compounds. The term "pressurized hot water" is used to denote the region of condensed phase of water in the range of temperature between 100 °C (boiling point of water) and 374 °C (critical point of water), where the pressure is regulated in such a way that water remains in its liquid state [9]. Under sub-critical conditions, the water's dielectric constant can be tuned by changing the temperature which in turn changes the water polarity. For instance, under standard temperature and pressure (25 °C and 101 kPa) water is a polar compound with dielectric constant of about 80 [10]; but, when the temperature is increased from 200 to 350 °C, the dielectric constant drops to around 20–30, which is similar to the range of dielectric constants of conventional solvents like methanol, ethanol and acetone at room temperature. Thus, at sub-critical conditions water behaves like certain organic solvents with the capacity of dissolve a wide range of medium and low polarity compounds. Moreover, it has been observed that the addition of some organic solvents, such as ethanol, can modify the dielectric constant of water – and its polarity –, and enhance the solubility of target analytes [10].

The feasibility of SWE as extraction method to obtain phenolic components from numerous kinds of vegetable matrices has been reported extensively [9–13]. However, to our knowledge, there are no reports on the use of this methodology to extract phenolics from peanuts. This study was aimed to evaluate the efficiency of water-ethanol at high pressure and temperature conditions to recover phenolic compounds from peanut skin. Theoretical models were tested against experimental data in order to optimize extraction conditions and antioxidant activity of target compounds.

2. Materials and methods

2.1. Plant material

Peanut skins were obtained from Runner-type peanuts by means of a typical industrial blanching process (90 °C, 10 min). This material was sieved using an automatic screen (EJR 2000 Zonytest, Argentina) to remove small particles of kernel, and then was fully defatted in a Soxhlet device (Soxhlet extractor 500 mL capacity, IVA, Argentina) using *n*-hexane as extraction solvent. The defatted material was milled using a universal cutting mill (Moulinex, France), and sieved to obtain uniform particle size (mean value 0.5 mm). The selected material was stored in amber glass containers at −20 °C under nitrogen atmosphere until use.

2.2. Sub-critical water extraction

Sub-critical water extraction (SWE) was carried out in an in-house developed apparatus according to the experimental setup reported in Barrera Vázquez et al. [14]. It consists of a stainless steel high-pressure extractor cell (18.5 mL internal volume), a HPLC pump (ELDEX, model OPTOS 2SM, California, USA) having a maximum flow rate of 10 mL/min, a coiled preheated, and a downstream back pressure regulator. The extraction cell is equipped with aluminum heating jackets with two electrical resistances and connected to a temperature regulator. To maintain the set temperature, the cell is mounted within a thermally insulated box. The pressure in the extractor was maintained at 7 MPa and measured with a pressure gauge (Dynisco Dynipack 16, Massachusetts, USA). The experimental apparatus is completed with stainless steel 1/8′× connecting lines and accessories. For each experimental run, a stainless steel membrane cartridge filled with 0.5 g of defatted peanut skin (DPS, obtained as described previously) was placed into the extraction cell. The temperature was set and the solvent volumetric flow was regulated by the HPLC pump to obtain temperature and solvent mass flow at the desired extraction conditions. The solvents used were distilled water and ethanol 95° (95%, v/v) (Porta, Argentina). For each run, a final extract volume equivalent to 150 g was collected, centrifuged at 10,000 rpm for 20 min, and stored in amber glass bottles under nitrogen at −20 °C until analysis.

2.3. Extract analyses

Total phenol content (TPC) was determined using the Folin-Ciocalteau reagent according to Singleton et al. [15]. The TPC from peanut skin extracts (PSE) obtained at the different SWE conditions was quantified by comparison of the absorbance value (725 nm) with those from a standard curve using gallic acid (GA), and expressed as gallic acid equivalent (mg GAE/g DPS, DB).

Total flavonoid content (TFC) was determined according to Larrauri et al. [5]. A 1 mL-aliquot of methanolic 2% (w/v) aluminum chloride was added to an aliquot (200, 500 or 1000 μL) of each PSE. After 10 min incubation at room temperature, the absorbance at 367 nm was measured. TFC was determined by comparison of the absorbance value with those from a standard curve using quercetin (Q), and expressed as quercetin equivalent (mg QE/g DPS, DB).

2.4. Radical scavenging capacity (RSC)

Radical scavenging capacity of each PSE was analyzed by means of DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) and hydroxyl radicals (DPPH·, ABTS·+ and HO·, respectively) assays.

Scavenging capacity against DPPH· was assessed according to Larrauri et al. [5]. Three concentrations of each sample extract were added separately to 1.5 mL DPPH· methanolic solution (20 μg/mL), and the absorbance of each mixture was determined after 5 min of mixing using a UV–vis spectrophotometer at 517 nm. RSC was estimated by means of the following equation:

$$RSC(\%)=\left[1-\frac{(absorbanceofDPPHandSample-absorbanceofSample}{absorbanceofDPPH}\right]\times 100 \quad (1)$$

Scavenging capacity against ABTS·+ was determined as described by Sarkis et al. [16], with some modifications. ABTS radical was produced by mixing an ABTS solution (7 mM) with potassium persulfate (2.45 mM), and keeping the mixture in the dark at room temperature for 12 h. Afterwards, the ABTS solution was diluted in ethanol 95% until absorbance at 734 nm reached 0.7 (± 0.02). Aliquots from 3 to 50 μL of each PSE were mixed with 1 mL of the ABTS· solution, and absorbance was determined after 6 min.

The HO· scavenging capacity was investigated following the procedure of Boiero et al. [17]. The reaction was performed in 50 mM phosphate buffer (pH 7.4) containing 10 mM deoxyribose, 100 mM H_2O_2, 1 mM $FeCl_3$, and 5 mM EDTA (ethylenediaminetetraacetic acid) in the presence and absence of the extracts at different concentrations. The reaction started with the addition of ascorbic acid in a final concentration of 5 mM. The reaction mixture was incubated for 1 h at 37 °C in a water bath. Then, 1% (W/V) TBA (thiobarbituric acid) and 2.8% (W/V) cold TCA (trichloroacetic acid) were added and heated to boiling temperature (95–100 °C) for 20 min to allow the coloured adduct to form, of which the absorbance was measured at 532 nm.

The RSC of PSE against ABTS·+ or HO· was calculated using the

following equation:

$$RSC(\%)=\left[\frac{(absorbanceofcontroland-absorbanceoftestsample}{absorbanceofcontrol}\right]\times 100 \quad (2)$$

For all RSC assays, the inhibitory concentrations were calculated as IC_{50} values (extract concentration which causes 50% decrease of the initial concentration of the corresponding radical). A lower IC_{50} value indicates higher antiradical activity.

2.5. Experimental design and response surface analysis

The experimental design for bioactive compound extraction from DPS was carried out using Response Surface Methodology (RSM). A Box-Behnken design with three factors was selected to identify relationships between the response variables and the process parameters, as well as those conditions that optimized the extraction process [18]. The response variables selected were TPC (mg GAE/g DPS) (Y_1), TFC (mg QE/g DPS) (Y_2), $IC_{50(DPPH)}$ (μg DPS/mL) (Y_3), $IC_{50(ABTS)}$ (μg DPS/mL) (Y_4) and $IC_{50(OH)}$ (μg DPS/mL) (Y_5). The independent variables were temperature (X_1: 140, 180 and 220 °C), solvent flow (X_2: 3, 5 and 7 g/min), and concentration of ethanol as extraction co-solvent (X_3: 0, 50 and 95%, v/v). The values for each of these three factors were based on preliminary experimental studies (data not shown). The design consisted of fifteen randomized runs including three replicates at the central point (Table 1).

Quadratic polynomials were fitted to express the responses (Y_n) as a function of factors; where Y is the response, β_0 is the constant term, β_i represents the coefficients of the linear parameters, X_i represents the factors, β_{ii} represents the coefficients of the quadratic parameter, and β_{ij} represents the coefficients of the interaction parameters.

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum\sum_{i<j}^{3} \beta_{ij} X_i X_j \quad (3)$$

The experimental results were analyzed (ANOVA test) to obtain the regression models. The fitness of the models was evaluated through both the determination coefficient (R^2) and the lack-of-fit test which explain the extent of the variance in a modelled variable that can be explained with the model. All determinations were performed in triplicate, randomly, and replicas of the central point were done to allow estimation of pure error as square sums. Statistical analyses were performed using Statgraphic Plus software (v5.1, USA).

2.6. Model validation and analyses of extracts under optimal extraction conditions

For model validation, the investigated response variables were tested under optimal extraction conditions. Triplicate runs were carried out. Composition of the obtained extracts was determined by means of HPLC-ESI–MS/MS analysis. Their RSC (DPPH·, ABTS·+ and HO· assays) was evaluated at different concentrations and compared with values obtained from the synthetic antioxidants BHT and Trolox (2-deoxy-D-ribose, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). The antioxidant activity was also tested in oil in water emulsion system (β-Carotene-linoleic acid bleaching test).

2.6.1. HPLC-ESI–MS/MS analysis

Phenolic composition of PSE was analyzed using an Agilent 1200 Series system (Agilent Technologies, Santa Clara, CA, USA) equipped with a gradient pump (Agilent G1312 B SL Binary), solvent degasser (Agilent G1379 B), and auto-sampler (40 μL sample loop, Agilent G1367 D SL + WP). The chromatographic separation was achieved on a Kromasil (Bohus, Sweden) reverse-phase C18 column (5 μm, 250 mm × 4.60 mm i.d.) according to Di Paola Naranjo et al. [19]. The column temperature was thermostated at 35 °C using a column heater module (Agilent G1316 B). The mobile phase consisted of 0.5% formic acid in ultrapure water (v/v, solvent A) and 0.5% formic acid in methanol (v/v, solvent B), starting with 20 and changing to 50% B during 3 min, kept for 5 min, followed by a second ramp to 70% B in 7 min, maintained for 5 min, a third ramp to 80% B in 1 min, maintained for 9 min, remaining at this last condition for 10 min before next run. The flow rate was 0.4 mL/min. The HPLC system was connected to a photodiode array detector (Agilent G1315C Satarlight DAD) and subsequently to a micrOTOF-Q11 Series QTOF mass spectrometer (Bruker, Billerica, MA, USA) equipped with electro spray ionization (ESI) interface. UV–vis spectra were registered from 200 to 600 nm. Mass spectra were recorded in negative ion mode between m/z 50 and 1000. The working conditions for the ionization source were as follows: capillary voltage, 4.500 V; nebulizer gas pressure, 4.0 bar; drying gas flow, 8.0 L/min; and drying gas temperature, 200 °C. Nitrogen and argon were used as nebulizer and collision gases, respectively. The MS detector was programmed to perform a MS/MS scan of the three most abundant ions, using collision energy of 13.0 eV.

Tentative identification of phenolic compounds was based on their

Table 1
Box–Behnken experimental design with SWE conditions and experimentally obtained values of total phenol content (TPC), total flavonoid content (TFC), and antioxidant activity (IC_{50}).

Run order	Independent variable			Investigated response				
	X_1 Temperature (°C)	X_2 Solvent flow (g/min)	X_3 Ethanol (%)	Y_1 TPC (mg GAE/g DPS)	Y_2 TFC (mg QE/g DPS)	Y_3 $IC_{50(DPPH)}$ (μg DPS/mL)	Y_4 $IC_{50(ABTS)}$ (μg DPS/mL)	Y_5 $IC_{50(OH)}$ (μg DPS/mL)
1	180	7	95	67.84 ± 0.00	12.00 ± 0.31	24.76 ± 1.00	55.72 ± 2.96	23.07 ± 0.003
2	220	7	50	164.79 ± 2.76	138.30 ± 0.31	9.33 ± 0.005	16.21 ± 0.69	16.52 ± 0.47
3[a]	180	5	50	95.61 ± 0.33	40.74 ± 2.17	10.81 ± 0.61	29.67 ± 0.75	14.65 ± 0.51
4	140	3	50	74.02 ± 2.46	16.18 ± 1.67	17.97 ± 0.84	21.93 ± 0.02	50.91 ± 0.35
5	140	7	50	68.02 ± 6.35	10.82 ± 0.13	18.76 ± 0.39	41.53 ± 1.51	46.07 ± 0.68
6	220	3	50	161.09 ± 2.78	154.98 ± 6.78	9.84 ± 1.01	19.31 ± 0.05	58.72 ± 0.72
7	140	5	95	48.67 ± 4.00	6.80 ± 0.55	30.05 ± 1.68	76.73 ± 2.46	28.62 ± 0.01
8[a]	180	5	50	122.18 ± 4.72	20.13 ± 9.46	10.25 ± 0.15	16.40 ± 0.002	15.43 ± 0.50
9	180	3	95	81.12 ± 4.22	14.38 ± 0.18	22.27 ± 0.69	31.69 ± 0.87	29.58 ± 0.04
10[a]	180	5	50	123.24 ± 1.46	54.22 ± 3.05	11.44 ± 0.75	30.69 ± 0.54	26.34 ± 0.25
11	180	3	0	57.49 ± 0.24	32.46 ± 1.34	40.27 ± 0.48	38.76 ± 1.74	39.65 ± 0.07
12	180	7	0	79.51 ± 4.78	41.34 ± 0.37	28.57 ± 0.19	39.77 ± 0.33	477.32 ± 30.79
13	220	5	0	49.24 ± 0.24	71.53 ± 0.61	45.80 ± 0.97	74.66 ± 1.67	1259.37 ± 7.25
14	220	5	95	102.55 ± 2.20	62.68 ± 0.15	19.11 ± 0.22	30.98 ± 0.15	19.29 ± 0.08
15	140	5	0	29.08 ± 2.29	8.05 ± 0.80	69.03 ± 0.18	83.53 ± 1.91	143.18 ± 3.94

Abbreviations: TPC, total phenol content; TFC, total flavonoid content; DPS, defatted peanut skin; GAE, gallic acid equivalent; QE, quercetin equivalent. Radical scavenging capacity is expressed as IC_{50} values; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical; OH, hydroxyl radical.

[a] Central point. Data from the investigated responses are expressed as arithmetic mean ± standard deviation (n = 2).

retention times (R*t*), elution order, UV–vis spectra and MS fragmentation spectra as compared with phenolic standards, in addition to those reported in the literature [4,5,8,20,21]. For this purpose, commercially available standards (caffeic and ferulic acids, and (+) catechin from Extrasynthèse, Genay, France; *p*-coumaric acid and quercetin from Fluka, United Kingdom; chlorogenic acid, rutin and *trans*-resveratrol from Sigma–Aldrich, Steinheim, Germany; gallic acid from Riedel-de-Haën, Seelze, Germany) were used. The Compass version 3.1 software and DataAnalysis version 4.1 software were used for data acquisition and processing respectively.

2.6.2. β-Carotene-linoleic acid bleaching test

This method is based on spectrophotometric measurements of β-carotene bleaching induced by the oxidative degradation products of linoleic acid. Briefly, 0.2 mg β-carotene in 1 mL chloroform, 20 mg of linoleic acid and 200 mg of Tween 20 (Polyoxyethylene sorbitan monolaurate) were transferred into a round-bottom flask. Once the chloroform was removed in a rotary evaporator at 35 °C, 50 mL distilled water was added and the resulting mixture was stirred vigorously. An aliquot of 4 mL of the emulsion were transferred to tubes containing the DPS extract in different concentrations (20, 50, 100, 150 and 200 μg/mL) or standard (BHT and Trolox) at 200 μg/mL. After mixing, the absorbance (470 nm) at zero (A^0) time was recorded. The remaining sample was placed in a water bath at 50 °C for a period of 2 h and the absorbance (A^{120}) was measured at 15 min intervals. A mixture, prepared as described previously, without β-carotene, was used as a blank. All determinations were carried out in triplicate. The data were expressed as antioxidant activity (AA%) calculated according to the following equation:

$$AA\% = \left[1 - \frac{A^0_{Sample} - A^{120}_{Sample}}{A^0_{Control} - A^{120}_{Control}}\right] \times 100 \quad (4)$$

2.7. Extraction kinetic models

Recovery of phenolic compounds under the optimal extraction condition, calculated by means of the polynomial equation given by RSM, was analyzed as a function of time. Extractions were performed during a total time of 105 min. For all fractions obtained, TPC and $RSC_{(DPPH)\%}$ values were determined as indicated previously. Furthermore, two extraction kinetic models, namely one-site and two-site kinetic desorption models [22], were compared to describe phenolic compound extraction at optimal conditions. One-site kinetic model describes the extractions that are controlled by intra-particle diffusion. This model assumes that initially the solute is uniformly distributed within the matrix. The calculation of the total mass of extracted solute (m_t) as a function of time (t) is given by the following equation:

$$\frac{m_t}{m_0} = 1 - \exp(-kt) \quad (5)$$

where m_0 is the initial mass of solute in the vegetable matrix and k is first order rate constant.

Two-site kinetic model is a modification of one-site desorption kinetic model, and assumes that a certain fraction of the solute (f) desorbs at faster rate, defined by first order rate constant k_1, and a remaining fraction (1-f) desorbs at slower rate, given by first order rate constant k_2.

It can be assumed that plant matrix is composed of vegetable cells where the solutes of interest are contained. When vegetable matrix is mechanically milled some cells are broken whereas others remain intact. The solute that desorbs at faster rate is related to the solute that is directly exposed to the extraction solvent due to broken cells and is easily extracted. This fraction of solute is known as “free solute”. On the other hand, the solute that remains inside the intact cells is more difficult to extract due to high mass transfer resistance inside the particle. This solute is known as “tied solute”, and is related to the slow rate of extraction [23].

The mathematical expression of two-site kinetic model used to calculate the total mass of extracted solute as a function of time is the following:

$$\frac{m_t}{m_0} = 1 - [f \exp(-k_1 t)] - [(1-f)exp(-k_2 t)] \quad (6)$$

3. Results and discussion

3.1. Fitting the response surface models

Experimental values from each of the investigated response variables (dependent variables) are reported in Table 1. Statistical analyses indicated that all of them fitted well to second order polynomial equations. For the response variables namely TPC (Y_1), TFC (Y_2), $IC_{50(DPPH)}$ (Y_3), and $IC_{50(ABTS)}$ (Y_4) the determination coefficients (R^2) were found to be 88.98, 87.94, 93.25 and 90.37, respectively; and the values from the lack-of-fit test (0.2817, 0.2376, 0.2360 and 0.2725, respectively) were not significant ($p > 0.05$). This latter suggests that the models were adequate for the observed data at a confidence level of 95%. So, all these variables were included in SWE optimization. For the response variable $IC_{50(OH)}$ the value from the lack-of-fit test was significant ($p = 0.004$, $R^2 = 81.01$).

3.2. Influence of extraction parameters on the investigated response variables

Fig. 1 shows the response surface graphs for the investigated response variables. They were plotted in function of two factors (independent variables) while the third factor was kept constant at middle level.

Regarding TPC, the effects of both the extraction temperature and the ethanol concentration were significant (p values equal to 0.0282 and 0.0247, respectively); the former in first-order linear effect (X_1), and the latter in second-order quadratic effect (X_2^2). None interactive effect was observed. The experimentally measured values varied from 29.08 to 164.79 (mg GAE/g DPS) (Table 1). The maximum was reached at the highest temperature tested (220 °C) using 50:50 (v/v) ethanol:-water ratio. At this solvent ratio, a decrease in temperature had a strong effect on phenolic compound recovery (Table 1, Fig. 1a). Extractions with pure water at the lowest temperature (140 °C) showed minimal TPC. The effect of solvent flow was negligible; nevertheless, extraction was slightly improved at the highest (7 g/min) flow rate.

Reported values for TPC from peanut skins varied widely depending mainly on the peanut processing method (for example blanching or roasting processes), and methodology for phenolic compound extraction. Considering peanut skins obtained from typical blanching processes, our results show maximum TPC (164.79 mg GAE/g DPS) higher than those reported elsewhere. Yu et al. [4] found that extracting blanched peanut skins overnight with 80% ethanol (room temperature) led to poor phenol recovery (15.1 mg GAE/g skin). Nepote et al. [24], however, reached a maximum TPC (118 mg/g skin) with 70% ethanol, at room temperature. Ballard et al. [25] reported a maximum predicted TPC of 143.6 mg GAE/g skin from blanched peanuts using microwave-assisted extraction under the optimized conditions of 30.8%, 30.9 °C and 12.2 min for ethanol concentration, temperature and time, respectively.

At temperatures higher than 200 °C, viscosity and surface tension of water are reduced and, at the same time, diffusivity characteristics are increased. These conditions may enhance water extraction capacity. Nevertheless, under the SWE conditions used here, it is clear that pure water is not effective for peanut polyphenol extraction. Mixtures containing similar amounts of both water and ethanol were the most

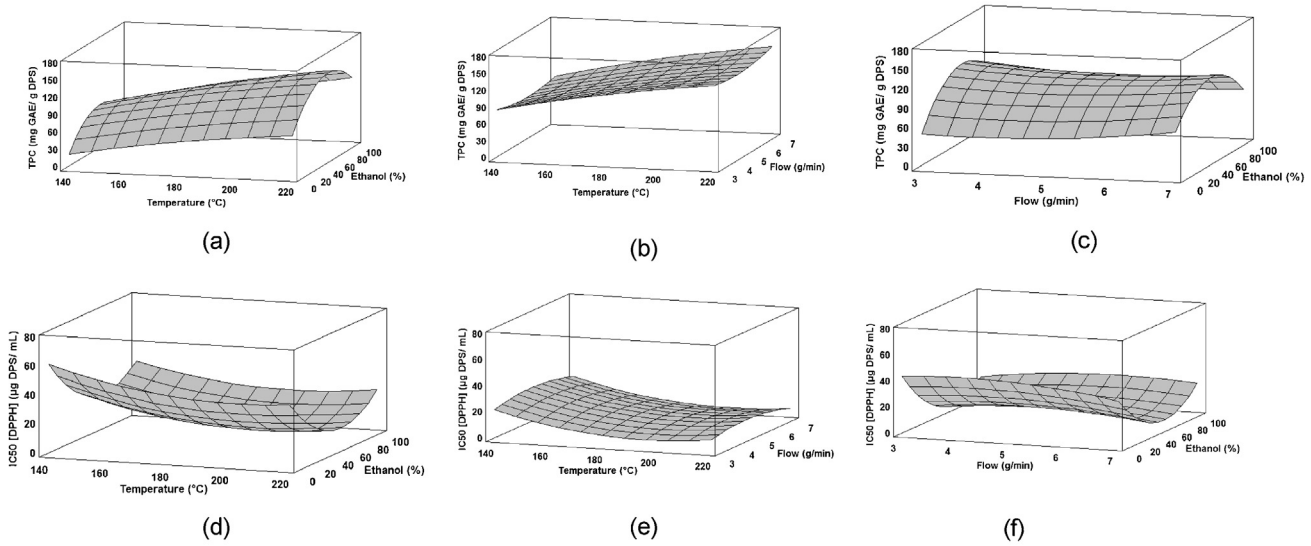

Fig. 1. Response surface plots showing combined effects of temperature (°C), solvent flow (g/min) and ethanol concentration (%) on total phenol content (TPC) (a–c), and IC_{50} (DPPH) value (d–f).

effective for extraction of these target compounds, but results were strongly dependent from the temperature.

Another factor that should be considered is the solvent polarity. Water is a very polar solvent (polarity index 9, dielectric constant 78.5, at 25 °C). According to data reported elsewhere [10], the highest temperature we tested (220 °C) could not be sufficient to reduce significantly the water's dielectric constant, i.e. its polarity. This fact may limit its extraction capacity to polar or very polar compounds. Ethanoĺs polarity (polarity index 5.2, dielectric constant 24, at 25 °C) is markedly lower than that of water. So, addition of ethanol to water may reduce polarity of this latter thus enhancing the capacity to dissolve a wider range of compounds, including those of medium polarity such as phenolics present in peanut skin.

The extracts obtained at the various SWE conditions showed TFC ranging between 6.80 and 154.98 mg QE/g DPS (Table 1). For this parameter only the effect of extraction temperature (X_1) was significant. Coincidently with results from TPC, the highest concentrations were achieved at the highest temperature tested, using 50% ethanol as co-solvent (Table 1).

The $IC_{50(DPPH)}$ and $IC_{50(ABTS)}$ values from extracts obtained at the various SWE conditions varied in the ranges 9.33–45.8 and 16.21–83.53 µg DPS/mL, respectively (Table 1). The similarity in the response surface plots indicates that conditions that enhanced DPPH· scavenging capacity (Fig. 1d–f) also favored ABTS·+ scavenging activity (data not shown). Ethanol concentration was the most important factor affecting these antioxidant activity-related parameters; temperature and solvent flow had minor effects. Notably, extractions using middle ethanol concentration (runs numbered 2,3,6,8 and 10, Table 1) had the highest and very similar $IC_{50(DPPH)}$ values (9.33–11.44 µg DPS/mL) despite they were done at middle (180 °C) or higher (220 °C) temperature. Correlations between TPC of extracts from the different SWE treatments and their RSC were significant for both $IC_{50(DPPH)}$ and $IC_{50(ABTS)}$ values ($r = -0.80$ in both cases).

3.3. Optimization and verification of mathematical models

Multiple graphical and numerical optimizations were run in order to determine the optimum levels of independent variables with desirable response goals. Predicted and experimental values for the investigated response variables at the optimal extraction condition are given in Table 2. According to the desirability function, the combination of variables for an optimal process that maximized extraction of both total phenolics and flavonoids, and gave the highest antioxidant activity, was 220 °C extraction temperature, 60.5% ethanol concentration, and 7 g/min solvent flow. Under these conditions, extractions were run in triplicate and averaged experimental values for TPC, TFC, $IC_{50(DPPH)}$ and $IC_{50(ABTS)}$ values were found to be 136.09 (mg GAE/g DPS), 103.80 (mg QE/g DPS), 10.52 (µg DPS/mL) and 17.05 (µg DPS/mL), respectively. No significant differences ($p > 0.05$) were found between each of these values and the corresponding predicted values thus suggesting good fit of the model to experimental data.

3.4. Extract composition and antioxidant activity under optimal extraction conditions

3.4.1. HPLC-ESI–MS/MS analysis

Twenty-four phenolic compounds were identified in DPS extracts obtained under optimum SWE conditions (Table 3). They primarily included different types of monomeric and condensed flavonoids, one phenolic acid (caffeic acid), and one coumarin derivative. In contrast with some previous studies [2,5,21,25], resveratrol (an stilbene derivative) was not detected.

Monomeric flavonoids included the flavanols catechin and epicatechin, two flavones (luteolin and chrysin), one flavonol (quercetin), and one *O*-methylated isoflavone (biochanin A). Luteolin and quercetin were also found as *O*-methylated derivatives (diosmetin and isorhamnetin, respectively). Flavonoid glycosides were almost absent; only one glycosilated isoflavone (3′5,7-trihydroxyisoflavone-4 -methoxy-3 -O-β -glucopyranoside) was identified as a minor component, in agreement with data reported previously [8,21]. The stereoisomers catechin and epicatechin were certainly identified by their typical $[M-H]^-$ signal at *m/z* 289, and a major MS/MS fragment at *m/z* 245; identity was also confirmed by *R*t matching with authentic commercial standards. These two flavan-3-ol derivatives were the most abundant monomeric flavonoids.

Overall, the phenolic profile was dominated largely by several isomeric forms of procyanidin dimers which were named with consecutive numbers according to their chromatographic *R*t. These oligomeric end products of the flavonoid biosynthetic pathway consisted exclusively of flavan-3-ol monomers (i.e., catechin and epicatechin), as it can be deduced by their typical $[M-H]^-$ signal at *m/z* 575, and major MS/MS fragments at *m/z* 285 and 449, in agreement with data reported elsewhere [20]. Differences among such oligomeric compounds are due to variations in stereochemistry and in the points of attachment (linkage) of catechin and epicatechin units each other [20,21]. Three proanthocyanidin dimers were also detected. Procyani-

Table 2
Predicted and experimental values at the optimal extraction condition (220 °C extraction temperature, 60.5% ethanol concentration, 7 g/min solvent flow).

	Extraction temperature (°C)	Solvent flow (g/min)	Ethanol concentration (%)	TPC (mg GAE/g DPS)	TFC (mg QE/g DPS)	$IC_{50(DPPH)(\mu g\ DPS/mL)}$	$IC_{50(ABTS)}$ (μg DPS/mL)
Predicted value	220	7	60.5	152.34	119.77	10.34	13.39
Experimental value	220	7	60.5	136.09 ± 0.23	103.80 ± 15.7	10.52 ± 0.93	17.05 ± 1.55

Abbreviations: TPCtotal phenol content; TFCtotal flavonoid content; DPSdefatted peanut skin; GAEgallic acid equivalent; QEquercetin equivalent. Radical scavenging capacity is expressed as IC50 values; DPPH2,2-diphenyl-1-picrylhydrazyl radical; ABTS2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical. Data are expressed as arithmetic mean ± standard deviation (n = 2).

Table 3
HPLC-ESI–MS/MS analysis of aqueous ethanol extracts obtained from peanut skins at the optimal extraction condition (220 °C extraction temperature, 7 g/min solvent flow, 60.5% ethanol as co-solvent).

Rt (min)	Compound	$[M-H]^-$ (*m/z*)	$MS^2[M-H]^-$ (*m/z*)	Relative percentage
9.9	Proanthocyanidin Dimer 1	573	262, 381, 531	0.94 ± 0.06
12.2	Proanthocyanidin Dimer 2	573	289, 453	0.41 ± 0.03
13.2	Catechin	289	203, 245	4.31 ± 0.46
13.3	Proanthocyanidin Dimer 3	573	289, 411	1.91 ± 0.25
13.5	Procyanidin Dimer 1	575	285, 411, 449	4.33 ± 0.85
13.6	Epicatechin	289	203, 245	3.53 ± 0.27
13.9	Procyanidin Dimer 2	575	285, 411	3.02 ± 0.24
14.5	Procyanidin Dimer 3	575	285, 449	12.32 ± 1.36
14.7	Procyanidin Dimer 4	575	285, 449	6.75 ± 0.90
15.0	Procyanidin Dimer 5	575	285, 449	12.71 ± 1.39
15.9	Procyanidin Dimer 6	575	285, 449	6.07 ± 0.95
16.4	Procyanidin Dimer 7	575	285, 449	10.20 ± 0.15
16.8	Procyanidin Dimer 8	575	285, 449	10.28 ± 0.42
17.0	Caffeic acid	179		Tr
17.3	Procyanidin Dimer 9	575	285, 449	10.93 ± 0.78
19.4	Procyanidin Dimer 10	575	285, 449	2.54 ± 0.48
20.3	Dihydroxycoumarin	177	133	0.56 ± 0.13
24.7	3′5,7-trihydroxyisoflavone-4-methoxy-3 -*O*-β-glucopyranoside	461	299	0.39 ± 0.05
25.4	Luteolin	285	199, 217, 241	1.16 ± 0.27
26.7	Quercetin	301	179	2.09 ± 0.38
29.5	Luteolin methyl ether (Diosmetin)	299	284	0.60 ± 0.18
30.4	Chrysin	253	209	0.18 ± 0.06
30.8	Quercetin methyl ether (Isorhamnetin)	315	300	1.02 ± 0.32
35.9	Biochanin A	283	268	1.82 ± 0.57

Compounds are listed on the basis of increasing retention times (Rt). Data are expressed as arithmetic mean ± standard deviation (n = 3).

dins and proanthocyanidins with higher degree of polymerization (e.g., trimers, tetramers and pentamers), reported previously as peanut skin components [4,8,20,26], were not found in the present study. The absence of higher molecular weight oligomers could be due to the particular conditions used in pressurized hot water extraction, as suggested by Herrero et al. [27] who point out the instability of certain compounds towards elevated temperatures as one of the major limitation of this extraction methodology. Thus, it is possible that at temperature and pressure conditions used in our study a direct cleavage of interflavan linkages might occur thus leading to prevalence of monomeric and dimeric flavonoids.

In general, the phenolic pattern we found, qualitatively and quantitatively dominated by procyanidin and proanthocyanidin oligomers, agrees with those obtained from peanut skin extracted with water or water-ethanol mixtures at normal pressure conditions. Examination of mature peanuts by Karchesy and Hemingway [28] showed that 17% by weight of skins were procyanidins consisting of low molecular weight oligomers. Similarly, Yu et al. [4] reported procyanidin dimers, trimers and tetramers as the major components in directly peeled peanut skin. On the other hand, Lou et al. [26] and Sarnosky et al. [20] isolated mostly oligomeric proanthocyanidins from the water-soluble fraction of peanuts skins.

3.4.2. Antioxidant activity

Further antioxidant activity assays were done to DPS extracts obtained under optimal extraction conditions. Fig. 2 shows data of RSC compared with those from BHT and Trolox, at different concentrations. In all assays tested (DPPH·, ABTS·+ and HO· scavenging capacity) peanut extracts had higher radical-inhibition percentages (better scavenging ability) than BHT. Notably, the maximum DPPH ··-inhibition percentage was reached at very low extract concentration, and it was approximately three fold higher than that of BHT. However, as compared with Trolox, peanut extracts showed similar (DPPH· assay) or lower (ABTS·+ and HO·) antiradical activities.

The antioxidant efficacy of phenolic compounds (measured as the free-radical scavenging capacity) primarily depends on the number of hydrogen-atom donor sites (typically hydroxyl groups attached to aromatic rings), but the position of these active groups is important as well. It is well-known that polyhydric phenols with a high number of

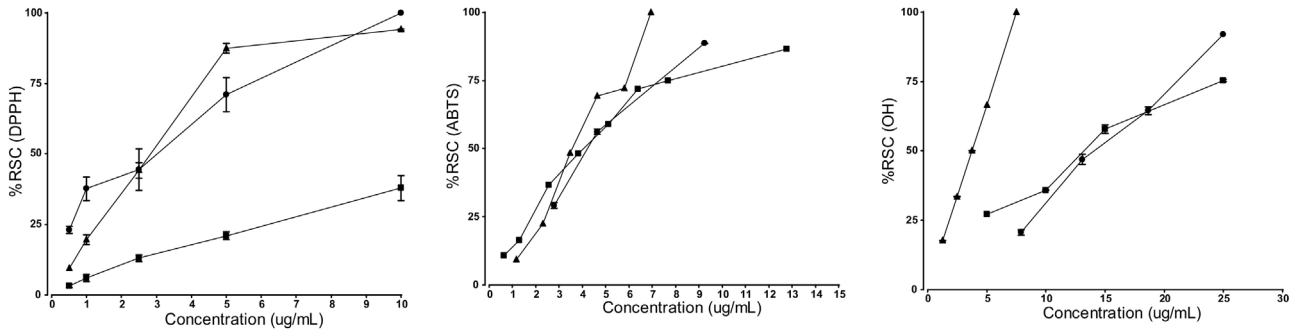

Fig. 2. Radical scavenging capacity from defatted peanut skin (DPS) extracts (●) obtained at the optimal extraction condition (220 °C extraction temperature, 60.5% ethanol concentration, 7 g/min solvent flow). The synthetic antioxidants BHT (■) and Trolox (▲) were used as references. Each point represents the arithmetic mean ± standard deviation (n = 3). Sub-figure references: (a) DPPH·, (b) ABTS·+, and (c) HO· scavenging capacity assays.

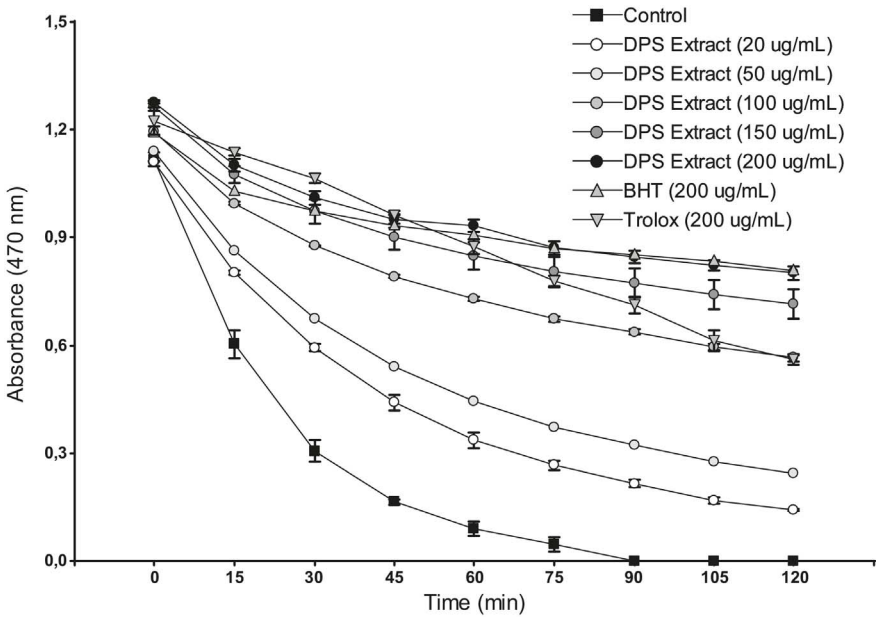

Fig. 3. Antioxidant activity (β-carotene-linoleic acid bleaching test) from defatted peanut skin (DPS) extracts obtained at the optimal extraction condition (220 °C extraction temperature, 60.5% ethanol concentration, 7 g/min solvent flow). Two synthetic antioxidants (BHT and Trolox), and a control sample without any antioxidant substance were used as references. Data are expressed as arithmetic mean ± standard deviation (n = 3).

Table 4
Antioxidant activity (β-carotene-linoleic acid bleaching test) from peanut skin extracts (PSE) obtained at the optimal extraction condition (220 °C extraction temperature, 60.5% ethanol concentration, 7 g/min solvent flow). Two synthetic antioxidants (BHT and Trolox) were used as references. Each point represents the arithmetic mean ± standard deviation (n = 3).

Antioxidant	Concentration (μg/mL)	AA%
PSE	20	35.91 ± 3.27
PSE	50	40.76 ± 0.50
PSE	100	58.35 ± 1.59
PSE	150	63.64 ± 1.78
PSE	200	68.56 ± 0.98
BHT	200	74.58 ± 0.50
Trolox	200	56.09 ± 3.25

Table 5
Kinetics of peanut skin phenolic extraction at the optimal extraction condition (220 °C extraction temperature, 7 g/min flow, 60.5% ethanol as co-solvent).

Extraction time (min)	TPC (mg GAE/g DPS)	Accumulated%	RSC (DPPH) %
0–10	110.96 ± 7.03	61.73	71.63 ± 4.01
10–20	35.53 ± 2.61	81.49	82.62 ± 0.50
20–30	14.13 ± 3.83	89.36	41.84 ± 7.02
30–45	9.09 ± 2.01	94.42	15.25 ± 5.52
45–60	4.03 ± 0.92	96.66	4.96 ± 2.01
60–75	2.22 ± 0.09	97.89	4.25 ± 0.01
75–90	2.03 ± 0.04	99.02	1.06 ± 0.50
90–105	1.75 ± 0.05	100	ND

Abbreviations: TPC, total phenol content; DPS, defatted peanut skin; GAE, gallic acid equivalent; RSC, radical scavenging capacity; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical; ND, Not detected. Data are expressed as arithmetic mean ± standard deviation (n = 3).

OH-groups, such as many compounds identified in peanut skin extracts, could have strong antioxidant activity in lipid peroxidation reactions owing to their capacity to hydrogen-atom transfer to lipid alkyl radicals. In addition, when polyphenolic substances are considered, *ortho*- and *para*-dihydric structures show better antioxidant activity because of their major ability to donate hydrogen atoms and to form quinone-type structures that are stabilized by resonance [29]. Looking at the structures of the identified compounds (Table 3) it can be seen that most of them have a high number of hydrogen-atom transfer sites. Considering the whole set of compounds, an average number of 6 phenolic-OH groups is found; the different isomers of both procyanidin and proanthocyanidin dimers have the highest ones. Moreover, with exception of chrysin, isorhamnetin and biochanin A, the identified phenolic compounds have at least one aromatic ring with two OH-groups as *ortho* substituents. Interestingly, Oldoni et al. [6] have reported that antioxidant activity of proanthocyanidins isolated from peanut skin extracts is considerably higher than those from the synthetic antioxidants BHA and BHT.

Table 6
Values for rate constants from both one-site and two-site kinetic desorption models obtained at the optimal extraction condition (220 °C extraction temperature, 7 g/min flow, 60.5% ethanol as co-solvent).

One-site kinetic model		Two-site kinetic model			
k (min^{-1})	AARD%	k_1 (min^{-1})	k_2 (min^{-1})	f	AARD%
0.09	2.21	0.12	0.03	0.82	0.46

Abbreviations: AARD, average absolute relative deviation calculated as indicated in Eq. (8).

Antioxidant activity of PSE, tested in an emulsion system (β-carotene-linoleic acid bleaching test), increased regularly with extract concentration (Fig. 3, Table 4). Notably, PSE showed higher antioxidant activity than Trolox, a hydrophilic synthetic antioxidant. At 200 μg/mL, the AA% of PSE equalled that reached by BHT (a lipophilic antioxidant). These facts suggest that peanut polyphenols could have good antioxidant activity in oil-in-water emulsions. This hypothesis is also supported by the structures of some of the identified compounds (Table 3) which are found to cover a wide range of partition coefficients [30], indicating that they could partition equally into both lipid and water phases.

3.5. Extraction kinetic at optimal operating conditions and kinetic model fitting

Table 5 shows TPC and RSC values as a function of the extraction time. Findings indicate that most phenolics can be recovered before

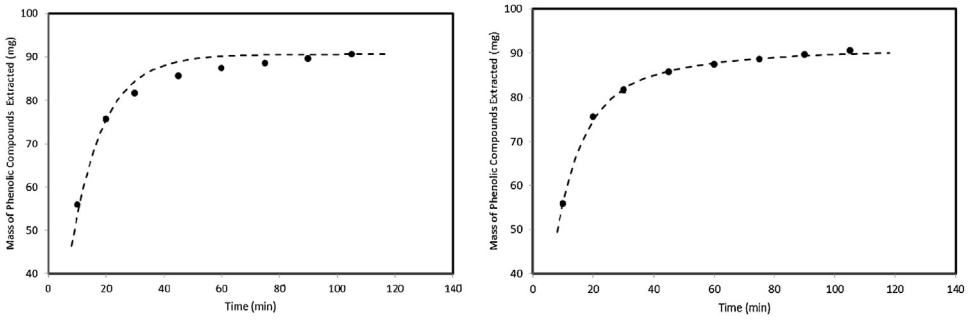

Fig. 4. Experimental extraction yields as a function of time (●) compared with those from proposed kinetic models (dash line). Sub-figure references: (a) One-site desorption kinetic model, (b) Two-site desorption kinetic model.

30 min extraction; negligible amounts are obtained later. Extracts achieved between 0 and 10 min and between 10 and 20 min had the highest scavenging capacity. All these facts suggest that extraction times shorter than 30 min may be sufficient to get concentrated extracts with good antioxidant activity; longer times may cause dilution of phenolic compounds in the final extract.

The kinetic parameters of both one-site and two-site desorption kinetic models were obtained by adjusting experimental extraction curve at optimal conditions using Generalized Reduced Gradient Non Linear (GRG Non Linear) algorithm from Microsoft Excel Solver. The objective function minimized was root mean square error (RMSE):

$$RMSE = \sqrt{\sum_{i=1}^{n} \frac{[(m_i/m_0)_{exp} - (m_i/m_0)_{model}]^2}{n}} \quad (7)$$

where "n" is the number of experimental data, and $(m_i/m_0)_{exp}$ and $(m_i/m_0)_{model}$ are dimensionless experimental extraction yield and the values of extraction yield predicted by the model, respectively.

Due to the different and numerous phenolic compounds that were identified in the extracts, phenolics were considered as a single solute, and TPC values collected at different operation times were used for calculation. The value of the initial mass of TPC in the vegetable matrix was estimated at 181 mg/g peanut skin. This amount of TPC was the maximum yield obtained for 105 min extraction operation at calculated optimal conditions. For this extraction time, an asymptotic value of accumulated TPC was achieved. The experimental conditions and the values for rate constants and parameters of both kinetic models are reported in Table 6. The goodness of the models was assessed comparing the average absolute relative deviation (AARD%) between experimental and predicted extraction yield:

$$AARD\% = \frac{1}{n}\sum_{i=1}^{n} \frac{(m_i/m_0)_{exp} - (m_i/m_0)_{model}}{(m_i/m_0)_{exp}} \times 100 \quad (8)$$

The results show that both models are able to predict experimental data with good accuracy. Nevertheless, two-site desorption kinetic model exhibits lower value of AARD% than one-site model. The small differences between predictions of both models could be attributed to the high value of the fraction of free solute estimated (f = 0.82). When "f" takes values close to unity, two-site kinetic model tends to one-site kinetic model.

Experimental values of extraction yield compared with proposed models are shown in Fig. 4. One-site kinetic model slightly overestimates extraction yield after 20 min of extraction. On the other hand, two-site kinetic model gives an accurate estimation of extraction yield in the complete interval of operation time.

4. Conclusions

The present research highlights the potentiality of using water-ethanol under sub-critical conditions to obtain antioxidant phenolic compounds from peanut skins, an undervalued by-product of peanut processing operations. Theoretical models were tested against experimental data in order to optimize extraction conditions and antioxidant activity of target compounds. Hydro-alcoholic mixtures showed to be effective as extracting solvents since they may scan a wide range of polarities regarding the compounds to be extracted. The maximum concentrations of total phenolics were achieved by using 60.5% ethanol as co-solvent, at 220 °C extraction temperature and 7 g/min solvent flow. Under these extraction conditions, a large number and variety of phenolic compounds were identified. Phenolic profile was dominated largely by monomeric and condensed flavonoids, particularly procyanidin and proanthocyanidin oligomers. Extracts obtained under those conditions also gave the best radical scavenging capacities, which were higher to those reached by using a synthetic antioxidant (BHT). Kinetic studies showed a high extraction rate of phenolic compounds until the first 30 min of extraction, and it was in parallel with the highest scavenging capacity. Two-site kinetic desorption model parameters were obtained for optimal operation conditions. This model was able to predict experimental data with very good accuracy. In summary, SWE proved to be an efficient, safety and inexpensive method, alternative to conventional extraction methods, such as traditional solid-liquid extraction, for phenolic compound recovery from peanut skins. In future studies, it is suggested that other process conditions (mainly temperature) be investigated to further improve extraction efficiency.

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